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# INVESTIGATION OF SELECTED SPECIES OF THE GENUS *ORGYIA* (LYMANTRIIDAE) USING ISOELECTROFOCUSING IN THIN LAYER POLYACRYLAMIDE GEL.

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## ABSTRACT

Egg-protein patterns from four selected species representing the genus *Orgyia* were obtained by electrofocusing in thin layer polyacrylamide gel. The patterns were specific and reproducible. The usefulness of this method in analysis of closely related species was suggested.

## INTRODUCTION

*Orgyia wardi* from Nova Scotia was described by Riotte (1971) as a distinct species from *Orgyia leucostigma* (J. E. Smith).

Both species occur sympatrically and even when they occupy the same tree there was no evidence of hybridizing. Initial separation of the two species was based on morphological differences of the eggs, the larvae, the pupae and the genitalia of both sexes, which were illustrated by Riotte (1971 and 1974). In the latter publication he further presented morphological characters of the larval mandibles, chaetotaxy of thoracic legs, female genitalia and electronmicrographs of the male genitalia.

Riotte (1973) also described *Orgyia definita rindgei* from New Mexico as new and a subspecies of the eastern *Orgyia definita* Packard. Subsequently this description, which was based upon a striking similarity of the larvae, was revised (Riotte, 1974), and it was suggested that *O. rindgei* could be a species *suo iure*. Riotte (1973) also indicated that the "final

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decision on the taxonomy of these populations will have to wait until detailed biological comparisons are complete."

It is apparent from the present study that egg protein patterns of *O. definita* and *O. rindgei* suggest that they are separate species, as are *O. wardi* and *O. leucostigma*. Colour illustrations of the larvae are also presented for comparison.

## MATERIALS AND METHODS

Eggs of *O. definita, leucostigma, rindgei* and *wardi* were collected from various places in North America in the spring, summer and winter of 1973. The egg masses were stored at —8C until ready for analysis. Thirty-five eggs were gently removed from the eggmass of each species using fine forceps under a dissecting microscope. The eggs were washed in distilled water and were homogenized in 200μl of 0.1 M Tris-HCl buffer pH 7.1 as described by Whitt (1970) using a small glass homogenizer. The homogenate was centrifuged in a Beckman 152 Microfuge at 8000 g for five minutes. The supernatant fraction was stored at —30C until subjected to electrofocusing in thin layer polyacrylamide gel using the LKB 2117 Multiphor. Detailed procedure for operating the Multiphor is already described in the LKB publication, Instruction Manual (1-2117-E 01), hence only modifications of the procedure such as the composition of the acrylamide gel formula will be described here. The gel was prepared by dissolving 4.25 g acrylamide and 0.20 g N, N'-Methylenebisacrylamide in 55 ml distilled water. The solution was filtered through Whatman no. 1 paper. Then 3.0 g sucrose, 15μl TMED (Gelling primer), 2 ml of 0.004% riboflavin, 20 mg of ammonium persulfate (gelling accelerator) and 3 ml of ampholine pH 3.5-10 (carrier ampholytes) were added into the solution. The unpolymerized acrylamide gel solution was poured into the gel moulding. After polymerization the gel was stored overnight in a container covered with a thin polyethylene sheet or SARAN Wrap at 4C. Before each run the gel was brought to room temperature and the sample was thawed. Using Eppendorf pipet 40μl of the sample was transferred onto Whatman 3MM paper (3x3x1 mm) placed on the cathodic region of the gel. Duplicate aliquots of each homogenate were used for the determination of the protein patterns. The thin layer gel electrofocusing was run at 10C for a total of 2.5 hours. The first 1.5 hours, constant current (25mA) was applied while the voltage

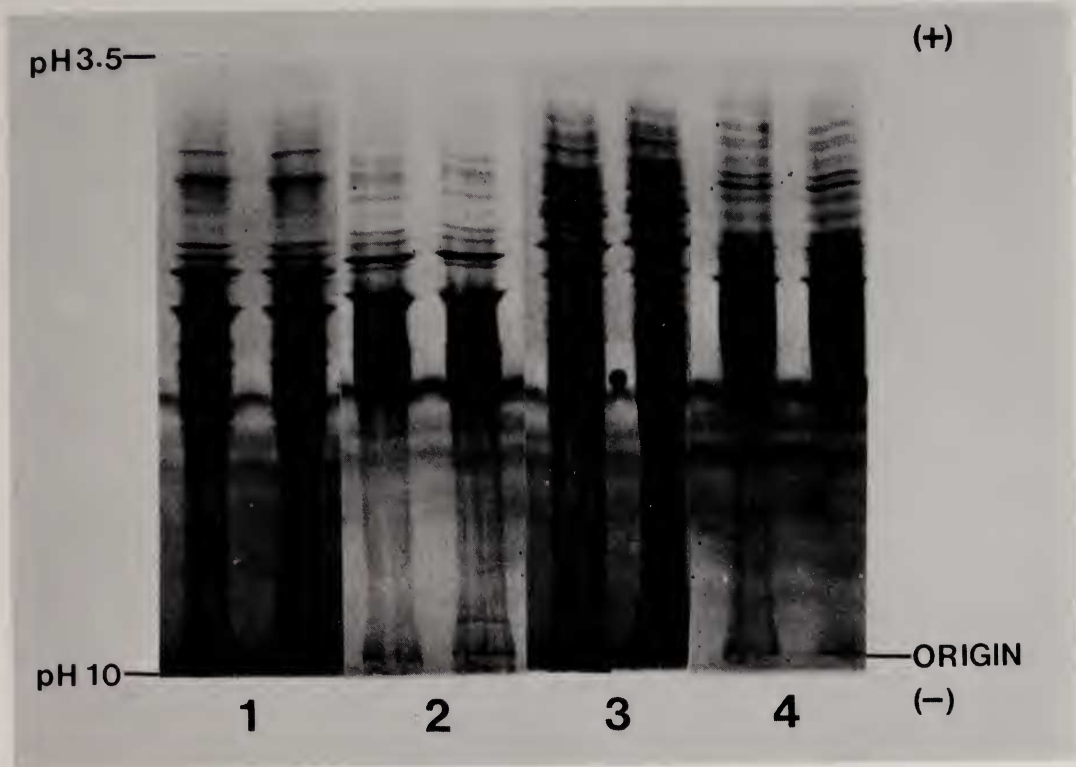


Fig. 1.—Electrofocusing patterns of soluble proteins from the eggs of: 1. *O. leucostigma*, 2. *O. wardi*, 3. *O. definita*, 4. *O. rindgei*.

Fig. 2.—Color illustrations of the larvae of: 1. *O. leucostigma*.

was allowed to increase from 70 to 800 volts. Then the run was changed to constant voltage (at 800 V) for the remaining one hour and the current was allowed to drop from 25 to 17 mA until the end of the run. Subsequently the gel was removed and stained and destained for general protein using Coomassie Brillian Blue R-250 as described by Karlsson et al. (1973). After twenty-four hours the bands were fully developed and the stained gel was photographed by reflected and transmitted light.

The larvae had been obtained from the same stock of eggs which were allowed to hatch in the laboratory at 22C.

## RESULTS AND DISCUSSION

Electrophoretic techniques have been used by many investigators for analysis of protein patterns for taxonomic purposes in insects (Stephen 1958, 1961; van Sande and Karcher 1960; Marty and Zolta 1968). In all cases the proteins of the hemolymph were used. However, hemolymph proteins are subject to variation because of differences in ontogenetic stage and environmental conditions of the insect donors (Laufer 1964 and Kock 1968). Salkeld (1969) in her studies of several insect taxa described and suggested the usefulness of newly laid eggs for determination of protein patterns using electrophoresis because the protein composition of such eggs is stable prior to differentiation of the embryo. Hence, in this investigation only newly laid eggs were subjected to the electrofocusing in thin layer polyacrylamide gel. This technique was chosen because it is easy to standardize and the proteins are separated according to a well defined physico-chemical constant, the isoelectric point (pI). Hence the protein zones are sharp and reproducible (Karlsson et al., 1974).

Fig. 1 indicates that there is an apparent distinction between the protein patterns of *O. leucostigma* (1) from that of *O. wardi* (2), both in the cathodic and anodic regions. The anodic protein bands of *O. definita* (3) and *O. rindgei* (4) also suggest specific differences between the two species. The investigation suggests that a high degree of species specificity and a good resolution of protein bands of insect eggs can be accomplished using the electrofocusing in thin layer polyacrylamide gel. Biochemical techniques such as this one can definitely complement and confirm other biological data in the field of analytical systematics.

2. *O. wardi.*

3. *O. definita.*

Fig. 2 illustrates the differences in coloration of the larvae of *Orgyia* species under investigation. The color dissimilarities of the larvae support the results of the egg protein determination using electrofocusing technique and vice versa.

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